Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

A 420
R31
33-63

December 1960

ARS-33-63

Cop-2

JAN 10 1961

PESTICIDE RESIDUES IN MEAT AND MILK

A Research Report

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

PESTICIDE RESIDUES IN MEAT AND MILK

A Research Report

By H. V. Claborn, Entomology Research Division, R. D. Radeleff, Animal Disease and Parasite Research Division, and R. C. Bushland, Entomology Research Division

The chlorinated hydrocarbon and some of the organophosphorus insecticides sprayed on cattle or sheep may be absorbed through the skin and stored in the fatty tissues. They also may be stored in the fat when present as contaminants of feed, and they may be excreted in the milk of dairy cows exposed to them in sprays or feed. Systemic insecticides (McGregor *et al.* 22) given orally or by injection can cause meat and milk contamination. Therefore, before an insecticide can be recommended for use on livestock or for the control of insects on pasture and forage crops, studies must be made to determine whether or not the treatments will lead to meat and milk contamination. If residues are produced, their extent and duration must be established.

Such studies have been conducted at the Kerrville, Tex., laboratory since 1948. This work has been carried out as a cooperative effort between entomologists, veterinarians, and chemists of the Agricultural Research Service.

Experimental Procedures

Selection of Animals and Feed

The different animals were of average range condition and in excellent health. All were maintained on balanced diets of grain concentrates and hay.

Particular care was taken to obtain only animals completely free of insecticide residues, either by using Government-owned livestock for which an accurate history was available, or by buying through reputable dealers. To further insure freedom from insecticides or other substances which might interfere with experimental processes, samples of omental fat or of milk were taken for analysis from each subject prior to treatment.

1/ This paper brings up to date the information in ARS-33-25 issued in July 1956.

Each shipment of feed received at the laboratory was immediately sampled and analyzed for any insecticidal content. Only insecticide-free feed was utilized.

Treatment of Animals and Feed

Dermal applications.--The spraying and dipping operations were carried out with emulsions or suspensions of an appropriate chemical. Suspensions were prepared from wettable powders supplied by the manufacturers, and emulsions from both laboratory and commercially supplied concentrates.

In spraying, each animal was wet as thoroughly as possible. In dipping, the entire animal was submerged.

Application to feed.--Feed was carefully weighed for each animal. The insecticide was applied in an acetone solution, the concentration adjusted so that 1 milliliter treated 1 pound of feed. The maximum period between applications of the insecticide and feeding was 16 hours. The animals were given food twice daily and any refused was weighed and accounted for.

Methods of Sampling

Milk.--In early studies, mechanical contamination of milk by small amounts of insecticide on the udder at the time of milking was considered. To check for this possibility, samples were drawn from the milk cisterns by use of metal cannulae and conducted through chemically clean tubing to the sampling vessel. No differences could be noted between samples drawn in this manner and those taken by milking after a careful washing of the udder. For regular milk sampling thereafter the udders were carefully washed and dried before attachment of the milkers. Milking was done by machine, the milk remaining in a sealed system until aliquots were withdrawn for analysis. Samples were held under refrigeration until analyzed.

Fat.--Fat samples were usually obtained from the omentum. The omentectomy technique used was that described by Radeleff (29); it permitted the removal of a control sample and several after treatment from the same animal. Some samples were obtained at the time of slaughter. They were placed in ice-cream cartons or polyethylene bags and frozen or refrigerated at 0°-5° C. until dehydrated, which required about 6 days.

The dehydrated fat was weighed, cut into small pieces, placed in a Waring Blendor with 125 ml. of the proper solvent, and blended for 2 minutes. Twenty grams of anhydrous sodium sulfate were added, and the mixture was blended for 1 minute longer. Then 1 gram of Filter Cel was added, mixed well, and the extract filtered. The blender and filter were washed with enough solvent to make a total volume of 200 ml. The filtered extracts were then treated for fat removal and analysis.

The methods of extracting the insecticides from milk were different from those used for the extraction from body fat. However, the butterfat was always removed with the insecticide, and the same method of fat removal and analysis were used in both determinations.

For DDT and TDE analyses chloroform was the solvent used in the extraction. In early experiments the removal of fat and determination of the insecticides were made by the method described by Schechter et al. (33). Later the method was modified so that larger fat samples could be used. This method is described by Jackson et al. (18).

For methoxychlor in fat, n-hexane was used in the extraction. The fat was removed and the methoxychlor determined by the method of Claborn and Beckman (5). In the first experiment methoxychlor in milk was determined by the method described by Prickett et al. (28), and in the second experiment by the method described by Claborn and Beckman.

For lindane analysis in earlier experiments, n-hexane was used for the extraction solvent and lindane was determined by the spectrophotometric method described by Davidow and Woodard (12). In later experiments chloroform was used for the solvent, the fat was removed with a mixture of fuming and concentrated sulfuric acid by the method described by Jackson et al. (18), and lindane was determined by the method described by Schechter and Hornstein (34).

For toxaphene, chlordane, gamma chlordane, heptachlor, dieldrin, aldrin, and endrin analyses, benzene was the solvent used for the fat extraction when the samples were analyzed for organically bound chlorine. The chlorine method described by Carter (3) was used in the early experiments; later (where the insecticides were recorded to 0.1 p.p.m.) more sensitive methods were used. Strobane, heptachlor, chlordane, and gamma chlordane were extracted from the fat with nitromethane, the organic chlorine was reduced with sodium, and the chlorides were titrated amperometrically with silver nitrate. The method is sensitive to ± 0.5 p.p.m. on a 25-gram sample of fat.

In the most recent experiments a combustion method developed by Hudy and Dunn (17) was used for determining total chlorine. A sample of fat was burned and the chlorides titrated amperometrically with silver nitrate. This combustion method, used in conjunction with a washing procedure to remove inorganic halides from butterfat and beef fat, proved to be the most satisfactory one tried. The complete removal of water-soluble materials greatly reduced the blank and the variability in control samples.

For estimation of the alkali-stable insecticides--aldrin, dieldrin, endrin, and heptachlor epoxide--by the chlorine method, the fat was saponified with alcoholic potassium hydroxide, the resulting soap solution extracted with n-hexane and the extract concentrated, burned, and the chlorides titrated with silver nitrate as described by O'Donnell and Agazzi (25). In later experiments the combustion step was replaced with sodium reduction.

Colorimetric methods for heptachlor epoxide, dieldrin, and aldrin were used in more recent experiments. For these methods, the same saponification and extraction procedure was used as for the total chlorine method, followed by chromatographic columns for further cleanup preceding color development. The method described by Meyer et al. (23) was used for the colorimetric determination of heptachlor epoxide except an alumina column was utilized instead of the activated Florex column. The methods for aldrin and dieldrin were described by O'Donnell et al. (26, 27).

Malathion was determined by a colorimetric method described by Norris et al. (24). The method is sensitive to ± 0.5 p.p.m. on a 20-gram sample of fat and to ± 0.02 p.p.m. on a 1000-gram sample of milk.

Perthane was determined by a spectrophotometric method developed by A. R. Weiss.2/

Dilan was determined by a colorimetric method developed by Jones and Riddick (20, 21) and modified by R. H. Cundiff.3/

Co-Ral was determined colorimetrically by a method developed by Claborn et al. (9). Before this chemical method was available some residue studies were made using P^{32}-labeled Co-Ral.

In addition to Co-Ral, other labeled phosphorus compounds on which residue studies have been made include ronnel, dimethoate, Bayer 22408, and Delnav.

No general method can be described that will be workable for all organophosphorus compounds, and recovery data must be obtained on any new compound under study. However, the following procedure gave good recoveries of the labeled phosphorus compounds from the body tissues and milk:

> The fat samples were extracted with n-hexane, and the insecticide separated from the fat by extracting the hexane solution with acetonitrile, the solvent removed and the residue dissolved in acetone and chromatographed using a short column of acid-washed alumina.

Muscle, heart, liver, and kidney tissues were dried by mascerating a 10-gram sample with 125 grams of anhydrous sodium sulfate and extracting with n-hexane. If necessary, the hexane solution may be extracted with acetonitrile and chromatographed through an alumina column with acetone to separate the insecticide from other phosphorus compounds or metabolites.

An alternate method for muscle, heart, liver, and kidney which permits the use of a larger sample was also used. The sample was blended with acetone. The mixture was spun in the centrifuge and the acetone decanted through a folded filter. The residue was blended again with n-hexane and filtered. The acetone extract was concentrated to a small volume, diluted

---

2/ Unpublished from Rohm and Haas Company.

3/ Unpublished from Commercial Solvents Corporation.

with water, and extracted with hexane. The combined hexane extracts were dried, concentrated to 100 ml., extracted with acetonitrile, and chromatographed through a column of acid-washed alumina.

Milk samples were diluted with an equal volume of alcohol and extracted with a 75:25 ethyl ether-n-hexane mixture. The extracts were washed with water, dried, and filtered. The solvent was removed as completely as possible by distillation and evaporation. The fatty residue was dissolved in n-hexane and extracted with acetonitrile and chromatographed as described above for fat separation.

It should be pointed out that the use of radioactive compounds for determining residues is not devoid of errors. If care is taken in extractions, the indicated residues will represent the maximum. Unless cleanup procedures are used which will remove all possible metabolites of the active element, the indicated residue may be higher than the actual. Thus, the results reported in the tables from experiments using P^{32}-labeled compounds may have been slightly higher than the actual residues present. However, since the most careful laboratory techniques were employed, it can be assumed that the values were accurate.

Delnav was determined by a colorimetric method described by Dunn (14).

The chemical formulations for the insecticides included in this paper which do not have common names are listed as follows:

American Cyanamid 12880 (O,O,-dimethyl S-(N-methylcarbamoylmethyl)-phosphorodithioate)

Bayer 22408 (O,O-diethyl O-naphthalimido phosphorothioate)

Co-Ral (O-(3-chloro-4-methylumbelliferone) O,O-diethyl phosphorothioate)

Delnav (2,3-p-dioxanedithiol S,S-bis(O,O-diethyl phosphorodithioate)

Dilan (1 part of 1,1-bis(p-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis(p-chlorophenyl)-2-nitrobutane)

Perthane (1,1-dichloro-2,2-bis(p-ethylphenyl)ethane)

Sevin (1-naphthyl methylcarbamate)

Strobane (a mixture of chlorinated terpenes with about 66% of chlorine)

Storage in Fat of Beef Cattle and Other Animals Receiving Spray Treatments

Single Treatments

As reported by Claborn et al.[4] groups of four 6-month-old calves (2 steers and 2 heifers) were sprayed with a xylene emulsion[5] of DDT, TDE, methoxychlor, or lindane. Fat samples were taken from all the animals before treatment and 2, 6, and 10 weeks after treatment; other samples were taken when necessary to determine the duration of fat contamination. The analytical results are shown in table 1.

TDE was stored in slightly smaller amounts than DDT. The storage of methoxychlor was only about one-fourth as great as that of DDT, and lindane was not detected in the fat at the concentrations used. The methoxychlor residues were eliminated in 10 weeks, but DDT and TDE were detected in significant amounts 27 weeks after treatment.

Lindane sprays and dips.--Four cattle were sprayed with an 0.075 percent emulsion of lindane, four cattle were dipped in the same formulation, four others were sprayed with the same concentration of a lindane suspension, and two were used for controls. Omental fat samples were taken by biopsy from all animals before treatment, and from half the animals from each group at weekly intervals after treatment for 8 weeks. The analysis of the fat samples is reported in table 2.

Six hogs were sprayed with a 0.06-percent lindane emulsion, six were sprayed with the same concentration of a suspension, and two untreated animals were kept for controls. Two hogs, one from each group, were slaughtered at 1, 2, 4, and 6 weeks after spraying and fat samples taken for analysis.

In the group sprayed with the suspension, the lindane residue in the fat was 0.47 p.p.m. 1 week after spraying, 0.14 p.p.m. 4 weeks after spraying, and none could be detected 6 weeks posttreatment. In the emulsion-sprayed group, the lindane residue in the fat was 0.66 p.p.m. 1 week after spraying but none was detected 4 weeks posttreatment.

Four sheep and four goats were dipped in a 0.025-percent suspension of lindane described by Jackson et al. (18). Fat samples were taken from all animals before dipping and from alternate pairs at 2-week intervals following treatment.

The lindane residue in the fat of the sheep averaged 4.22 p.p.m. 2 weeks after dipping, decreased to 0.26 p.p.m. at 8 weeks, and could not be detected at 12 weeks posttreatment.

---

[4] Unpublished report entitled "Contamination of Meat by Chlorinated Hydrocarbon Insecticides for Insect Control" by H. V. Claborn, H. F. Beckman, R. W. Wells, R. C. Bushland, R. D. Radeleff, and W. J. Nickerson.

[5] All xylene emulsifiable concentrates contained 25 percent (by weight) of insecticide, 65 percent xylene, and 10 percent Triton X-100.

Co-Ral sprays and oral treatments.--A residue study was made using P^{32}-labeled Co-Ral. Two calves were sprayed with different amounts of the radioactive insecticide and two other calves were given oral doses. Two animals were slaughtered 1 week after treatment and the other two were slaughtered 1 week later. The residues in the tissues indicated by the radiometric assay are shown in table 3.

In another experiment, cattle, sheep, and goats were sprayed with Co-Ral and slaughtered at various intervals after spraying. Several samples taken from the animals were analyzed by the colorimetric method. Residues of 0.40 and 0.26 p.p.m. of Co-Ral were found in the omental fat 1 week after spraying with 0.5-percent Co-Ral and 0.07 p.p.m. was found in the omental fat 2 days after the sixth weekly spraying with 0.25 percent. There were no detectable residues in the fat 2 weeks after spraying with 0.5 percent nor in fat, liver, kidney, or muscle tissues 4 weeks after 3 biweekly sprays with 0.5 percent. Tissues were also analyzed by the Chemagro Corporation using a photofluorometric method described by Anderson et al. (1). They agreed very closely with the results obtained at Kerrville.

There is good agreement in the results of this experiment and that with the P^{32}-labeled Co-Ral described above, except that the apparent residues in the kidney and liver indicated by radioactivity were higher than those by chemical analysis. This was probably because of some breakdown product containing phosphorus. These samples were not partitioned between n-hexane and acetonitrile as were the fat samples.

Delnav and dimethoate.--A calf was sprayed with 16.2 mg./kg. of P^{32}-labeled technical Delnav. The animal was slaughtered 7 days after treatment and tissues were taken for analysis. Results are shown in table 3. Analysis of fat from hogs, sheep, goats, and cattle 2 days after spraying with 0.25 percent of Delnav are shown in table 1. Results for dimethoate on calves and sheep are given in table 3.

Multiple Treatments

DDT, TDE, methoxychlor, and lindane.--Four groups of six calves (three steers and three heifers) were sprayed with one of these insecticides. The spray concentrations and formulations were the same as were used in the single treatments, but were repeated six times at intervals of 3 weeks. Samples of fat were taken from all six calves of each group before the first treatment, and from three calves of each group 3 weeks after the first, second, fourth, and sixth treatments and then at 12, 24, and 36 weeks after the last spraying. The results of analyses of fat samples are reported in table 4.

The storage of DDT and TDE following the second spray treatment was almost twice as great as that after the first treatment, but further spray treatments caused no significant increases. The values for methoxychlor were variable, but the highest value was obtained after the sixth spraying. The results with DDT indicated that the storage of the insecticide in the fat reached a maximum after six applications.

Extensive treatments with DDT.--An experiment described by Radeleff *et al.* (31) was run to determine the residues that would result from spray treatments over an extended period of time.

Six 6-month-old calves were purchased at a livestock market. Fat samples taken from these animals were analyzed. The DDT content ranged from 5 to 75 p.p.m. and averaged 45 p.p.m. The calves were then sprayed with 0.5-percent DDT emulsion five times at 3-week intervals and after 5 days were given 28 additional treatments, at 2-week intervals. The DDT content of the fat samples taken 2 weeks after the last treatment ranged from 76 to 89 p.p.m. and averaged 84 p.p.m. The one animal that had 75 p.p.m. at the start had only 81 p.p.m. after 33 experimental sprayings.

Another group of 6 calves, fat samples from which were negative for DDT before treatment, were sprayed 28 times at 2-week intervals. Fat samples taken from five of the animals 2 weeks after the last treatment contained from 80 to 103 p.p.m. of DDT, with an average of 88 p.p.m., or 4 p.p.m. more than for the first group.

Cows and calves sprayed with DDT.--To investigate residues in fat from the consumption of contaminated milk, eight Hereford cows with young calves were used. All the cows and four of the calves were sprayed five times at 28-day intervals with 0.5-percent DDT, half of these animals with an emulsion and the other half with a suspension. The other four calves were not sprayed. Each calf lived upon uncontaminated grass and milk supplied by its mother. All the animals were slaughtered 28 days after the last spraying.

An average of 52 p.p.m. of DDT was found in the fat from the calves that were getting DDT both by absorption from spraying and ingestion of milk and 25 p.p.m. from those that were getting DDT only from the milk. Thus, more DDT was stored in the fat of the calves that were not sprayed than in the fat of their mothers, which averaged only 15 p.p.m.

Dieldrin.--Three heifers were sprayed four times at 3-week intervals with 0.05-percent dieldrin. Analyses of fat samples taken after each spraying and 11 and 28 weeks after the last spraying are given in table 4.

Heptachlor, chlordane, and gamma chlordane.--Four calves were sprayed six times at 2-week intervals with a xylene emulsion containing 0.5 percent of gamma chlordane, and four other calves were sprayed in the same manner with an emulsion of heptachlor. A third group of four calves was used for controls. Fat samples for analysis were taken by biopsy from all animals before spraying; from two calves from each group 2 weeks after each of the first five sprayings; and from all animals 2, 8, and 16 weeks after the sixth spraying.

Four calves in good condition were sprayed 12 times at 2-week intervals with a 0.5-percent emulsion of chlordane. Four other calves in an emaciated condition were given the same treatment. Omental fat samples were taken before treatment and 2, 8, and 16 weeks after the last spraying.

The fat samples were analyzed by the total chlorine method, and the results are reported in table 4.

Toxaphene and Strobane.—Fat samples were taken from cattle sprayed 12 times at 2-week intervals with toxaphene applied at a 0.5-percent concentration. Six animals were sprayed with a xylene emulsion formulated at Kerrville and six with an emulsion prepared from a concentrate furnished by the Humble Oil and Refining Company. In each group biopsy fat samples were taken from all animals before the treatments--from three animals 2 weeks after each of the first six sprayings; from two 2 weeks after each of the last six sprayings; from one 4 weeks after the last spraying; and from two 2 weeks later. The analytical results are shown in table 5.

In a similar experiment, cattle were sprayed 12 times at 2-week intervals with 0.5-percent Strobane, 6 animals with an emulsion and 6 animals with a suspension. Fat samples were taken at indicated intervals after spray treatments. The analytical results are shown in table 6.

Six cattle were sprayed 6 times at 2-week intervals with 2-percent Strobane. Fat samples were taken before the first spray treatment and 6, 10, and 14 weeks after the last spraying. The fat analyses are reported in table 4.

Malathion.--Eight calves were sprayed 16 times at weekly intervals with 0.5-percent malathion, four with an emulsion and four with a suspension. Fat samples were taken before the first spraying and 2 weeks after the last spraying. No malathion was detected in any of the samples.

Two sheep, one shorn and the other in fleece, and two goats, one shorn and one in fleece, were sprayed twice, 1 week apart, with 1.0-percent malathion. One untreated sheep and one goat were kept for controls. All animals were slaughtered 1 week after the second spraying and samples of omental fat, renal fat, and lean meat were taken for analysis. Little, if any, malathion was detected in any of the samples as reported by Roberts et al. (32).

Delnav.--Cattle, sheep, goats, and hogs were sprayed with Delnav. Samples from these animals were analyzed by the colorimetric method. All of the samples were analyzed by Hercules chemists and a few of the samples were analyzed at Kerrville. The results of the analyses made at Kerrville are shown in tables 1 and 4. These results are in good agreement with the results reported by Hercules.

Storage in Fat of Beef Cattle and Other Animals Following Ingestion of Contaminated Feed

The great variation in the residues left on forage owing to methods of application, difference in growth rate of individual crops, and weather conditions made it difficult to determine what concentrations of insecticides would normally be encountered on forage crops. Residue data obtained by the Division's forage-insect entomologists were considered when these experiments were planned. In the first tests the insecticides were added to feed in dosages likely to exceed field contamination. If the storage seemed excessive, further tests were made at progressively lower dosages until the minimum field residues were studied.

The cattle were fed in individual stalls twice daily and were given sufficient feed to maintain good weight gains. The sheep were grouped as ewes or wethers and fed in groups of two or three; otherwise the feeding was the same as for the cattle. The sheep were kept in small pens throughout the test period, but the cattle were allowed freedom in an exercise lot when not being fed.

A few studies covered a feeding period of only 4 weeks. In others the time was extended to 8, 14, or to 16 weeks, which is the maximum time that cattle and sheep are kept on feed when being finished for slaughter. Samples were taken before the feeding period started, at 2- or 4-week intervals during feeding, and at indicated intervals after the feeding of the insecticides ceased. Some of the insecticides were fed to cattle only, others to both sheep and cattle. Some were fed at one level only and some at varying levels. Residue studies were made on aldrin, BHC, chlordane, DDT, dieldrin, endrin, heptachlor, methoxychlor, and lindane. The analyses of the fat samples are shown in table 7.

It has been shown by Bann et al. (2) that aldrin is metabolized within the body and is stored in the fat as dieldrin, and by Davidow and Radomski (13) that heptachlor is stored in the fat as heptachlor epoxide. We were not aware of these conversions at the time of our first experiments described by Claborn et al. (8), but since the calculations were made from total chlorine determinations, the residues reported were apparently correct. The correction from aldrin to dieldrin would increase the reported residues by 3.8 percent and the correction for heptachlor would increase the reported residues by 3.7 percent.

An experiment was carried out in which sheep, cattle, and hogs were fed different amounts of aldrin for a period of 12 weeks. Some of the animals were slaughtered at the end of the feeding period and some 6 weeks later. Fat, muscle, liver, and kidney tissues were analyzed for both dieldrin and aldrin by specific colorimetric methods; some were also analyzed for total chlorine.

Only small amounts of aldrin were found even at the highest feeding level of 10 p.p.m. Most of the aldrin was oxidized and stored as dieldrin. The results of the dieldrin analyses are shown in table 8.

The dieldrin residues were slightly less in the sheep than in the steers. The residue in the fat of the hogs was approximately one-half that in the steers.

The dieldrin in beef roast was not reduced significantly by cooking. A sample of fat taken from the roasting pan after 3 hours at 350° F. contained the same amount of dieldrin as uncooked fat.

The chlorine found in fat from the steers could practically all be accounted for by the dieldrin and aldrin present in the fat. Other chlorine metabolites of aldrin were not present in significant amounts.

In a feeding experiment, cattle were fed varying levels of heptachlor ranging from 1 to 60 p.p.m. for 16 weeks. Fat samples were taken by biopsy before feeding started and at 2-week intervals during the feeding period. Only a few fat samples were available for analysis at Kerrville. These were analyzed for heptachlor epoxide by a colorimetric method; the results are shown in table 9.

After it was demonstrated by Gannon and Decker (15) that heptachlor is oxidized to heptachlor epoxide on forage, an experiment was planned in which low levels of heptachlor and heptachlor epoxide were fed to cattle for comparison of the resulting residues. Fat samples were taken by biopsy and analyzed for heptachlor epoxide by the colorimetric method. The results, shown in table 10, indicate that residues from feeding heptachlor epoxide were about 10 times as great as those from the same level of heptachlor.

Methoxychlor was the only insecticide that did not cause some storage in the fat.

Contamination of Milk

Rate of Excretion of Different Insecticides from Spray Treatment

Since Howell *et al.* (16) discovered that DDT was excreted in the milk of cows sprayed with this insecticide, many studies have been made to determine the rate of excretion and the duration of the contamination. Less work has been done on the other chlorinated hydrocarbon insecticides because they have not been used so extensively on livestock or because methods of analyses of comparable sensitivity were not available.

During the last 7 years data have been accumulated for a comparison of the typical rates and duration of excretion after applications of sprays containing 0.5 percent of DDT, methoxychlor, Dilan, Perthane, malathion, toxaphene, Strobane, and dieldrin. Successful control against hornflies (*Siphona irritans* (L.)) was obtained with a 0.5-percent concentration of any of these insecticides except dieldrin and would have been practical if no residues had appeared in the milk. With dieldrin, a lower concentration was sufficient for control. In these experiments, however, dieldrin was also used at 0.5 percent so that rates of excretion could be compared equally.

Since all the insecticides used were fat-soluble and were known to be present only in the butterfat of milk, the butterfat of each sample was determined, and the results were adjusted to a uniform butterfat content of 4 percent. The milk samples were taken before spraying and at various intervals thereafter.

Control cows were used in all experiments. These cows received the same feed, and milk samples were taken from them at the same time they were taken from the treated cows. In all the experiments the samples from the control cows were negative.

DDT.--Although the excretion of DDT in milk following treatment of commercial dairy cattle was reported in 1950 by Claborn et al. (7), this work was repeated in order that the treatment would be uniform for all materials. Three cows were sprayed with a 0.5-percent suspension. The results are given in table 11 (Claborn and Wells 10).

Dieldrin.--One cow was sprayed with a 0.5-percent dieldrin emulsion (Claborn and Wells 10). The results, given in table 12, agree very closely with bioassay data obtained by Y. P. Sun[6] on milk samples from two other cows similarly treated.

Methoxychlor.--Carter et al. (4) found that the presence of methoxychlor in milk following spray treatments could not be definitely shown by the organic-chlorine method, which at the time of their study was the only one available. The development of a sensitive colorimetric method by Prickett et al. (28) made more precise determinations possible.

One cow was sprayed with a 0.5-percent methoxychlor emulsion and another cow with the same concentration as a suspension. The excretion of methoxychlor had reached zero at the end of 21 days, and the test was repeated on the same cows. Results of the analyses by Clayborn and Wells (10) are given in table 12. The four samples collected from these two cows averaged 0.42 p.p.m. 2 days after spraying and all were practically zero after 14 days. The average of all samples analyzed over the two 21-day periods was 0.19 p.p.m.

Following the studies with individually treated animals, experiments were conducted with herds. Previous experiments by Claborn et al. (7) with other insecticides indicated that lower residues resulted when groups of animals were sprayed with commercial equipment. Two herds of dairy cattle were sprayed at Kerrville with a 0.5-percent methoxychlor suspension of a wettable powder. Application was at a rate of 2 quarts per animal. Pooled milk samples from one herd showed 0.18, 0.13, 0.11, and 0.05 p.p.m. of methoxychlor to be present 1, 2, 3, and 5 days after spraying. On the 7th, 14th, and 21st days, less than 0.05 p.p.m. was found. Similar data were obtained from the other sprayed herd.

Dilan.--Two cows were sprayed twice 14 days apart with a 0.5-percent suspension of Dilan (Claborn and Wells 10). Milk samples were collected at different intervals during the 14 days after the 1st and 21st days after the second spraying. Results of the analyses are given in table 13.

---

[6] Unpublished report from Julius Hyman and Company.

Malathion.--Four cows were sprayed twice, 1 week apart, with malathion, two with emulsions and two with suspensions, each at 0.5- and 1-percent concentrations (Claborn *et al.* 11). Samples of milk were taken at different intervals and freeze-dried, most of them immediately after they were taken, the others within 24 hours. The cows excreted malathion ranging from 0.08 to 0.36 p.p.m. in all samples of milk taken 5 hours after the spraying. Traces were found in samples taken after 1 day, but after 3 and 7 days none could be detected. The higher concentrations caused heavier residues than the lower concentrations, and the suspensions caused heavier residues than the same concentrations applied as emulsions.

Perthane.--Four cows were sprayed twice, 3 weeks apart, two with an emulsion and two with a suspension; and two cows were sprayed twice daily for 21 days with 1 ounce of an oil solution. All the sprays contained 0.5 percent of Perthane. The milk samples were analyzed by chemists of Rohm & Haas Company, and duplicates from two of the cows were analyzed at Kerrville. The results of the analyses are shown in table 14. These results are confusing since the higher values appear at random instead of at some definite time after spraying. The variations, which were shown to be owing to a variable blank, clearly indicate the unreliability of the method. However, since the maximum value was lower than that for methoxychlor, it seemed to be evident that Perthane sprays caused less contamination of milk than methoxychlor sprays of the same concentration.

Toxaphene and Strobane.--Six cows were sprayed with toxaphene and six with Strobane, and two cows were used for controls. Of the treated cows, four were sprayed twice, 3 weeks apart, two with an emulsion and two with a suspension; and two were sprayed twice daily for 21 days with a 2-percent oil spray. The analyses of the samples from the emulsion- and suspension-sprayed cows are shown in table 15, and from the oil-sprayed cows in table 16. The maximum contamination occurred 1 and 2 days after spraying. There was no significant difference in contamination caused by the suspensions and emulsions, or by the two insecticides.

Rotenone.--Dairy cows sprayed with Cubé at five times the normal concentration used for cattle grub control excreted no detectable rotenoids in the milk. The samples were analyzed by an ultraviolet, spectrophotometric method and a paper chromatographic method with limits of sensitivity of 0.04 and 0.006 p.p.m. of rotenone or dehydrorotenone. The samples were analyzed by Jacobson *et al.* (19).

Co-Ral.--Two dairy cows were sprayed with P^{32}-labeled Co-Ral. One of the cows was sprayed with a concentration of 0.5 percent and the other with one of 0.75 percent (Radeleff and Claborn 30). Milk samples were taken 5 hours after spraying, and twice a day for the first week, and once a day for the following 18 days. The analyses of the samples are shown in table 17.

Excretion of Insecticides Resulting from Ingestion of Contaminated Feed

Sevin and dicapthon.--Each of these insecticides was fed to two dairy cows. One cow in each feeding group was fed the insecticide at dosages of 2.5 p.p.m. the first, 5 p.p.m. the second, and 10 p.p.m. the third week. The other cow in each group was fed at 10 p.p.m. the first, 25 p.p.m. the second, and 50 p.p.m. the third week. Milk samples taken during the feeding period were analyzed at Beltsville, Md. (Westlake, W. E., H. G. Wheeler, and R. T. Brown, unpublished.) For Sevin, samples were extracted with a 3:1 mixture of *n*-hexane and methylene chloride. Before extraction the cream was permitted to separate and the volume of the sample reduced to 150 ml. by siphoning off the milk. Three extractions were made each with 150 ml. of solvent. The analysis procedure was developed by the Union Carbide Chemicals Company. For dicaphthon, analyses were made by a process devised by the American Cyanamid Company. Milk samples were extracted by shaking with a 1:1 mixture of ether and *n*-hexane. For a 400 ml. volume of milk, 150 ml. of solvent was used. Neither Sevin nor dicapthon was detected in any of the samples.

Bayer 22408.--One dairy cow was fed the P^{32}-labeled insecticide at 5 p.p.m. in the diet for 7 days; when no residue appeared in the milk, the dosage was raised to 100 p.p.m. and the feeding continued for 12 days. Another cow was fed 50 p.p.m. of the material in the diet for 7 days. Milk samples were taken daily. Bayer 22408 was not detected in any of the samples. An extraction procedure, sensitive to 0.01 p.p.m., gave 75-80 percent recoveries when the insecticide was added to whole milk.

Toxaphene.--Toxaphene was fed to dairy cows as a contaminant of the feed at dosages of 20, 60, 100, and 140 p.p.m. for a period of 8 weeks. The insecticide was excreted in the milk at all the dosage levels, the parts per million appearing in the milk roughly 1 percent of the concentration fed. At the end of the first or second week the contamination of the milk had approached the maximum level. Slight increases were noted in the two lower feeding groups through the first 6 weeks, after which the contamination decreased. In the two higher feeding groups slight increases continued through the first 4 weeks, followed by a decrease at the end of the fifth week. There was a rapid decrease in the extraction of the insecticide in the milk 1 week after the feeding ceased. Further decreases were extremely slow. The chloride content of the butterfat from the two higher feeding groups had not decreased to normal after 3 weeks. The results of the analyses are shown in table 18.

Milk from Commercial Dairies in Which Barns and Cows Were Sprayed with DDT or TDE

During the summer of 1948, DDT and TDE were tested for fly control at seven commercial dairies by Claborn *et al*. (see footnote 4 on page 6). The insecticides were used as 5-percent emulsions for spraying barns and 0.5 percent for spraying the cattle. The barns were sprayed as often as needed for the control of house flies (*Musca domestica* L.), and the cattle whenever horn flies became annoying. All the test herds excreted detectable amounts of insecticide in their milk throughout the fly season (May to September). The average contamination was 0.21 p.p.m. for the four herds treated with DDT and 0.25 p.p.m. for the three treated with TDE.

Source of Contamination of Milk from Sprays Applied to Dairy Barns

A study was made at Kerrville by Claborn *et al.* (6) to determine the source of milk contamination when dairy barns were sprayed with 2.5 percent of DDT. Three experiments showed that (a) the insecticide was actually secreted in the milk and did not get into it from mishandling of the milk or equipment, (b) no contamination resulted from inhalation of the insecticide by the cows, and (c) the insecticides excreted in the milk came from residues left on the feed troughs. Contamination did not occur when feed troughs were completely covered during the spraying or were washed afterwards.

Residues in Tissues Resulting from Use of Systemic Insecticides

Ronnel.--A calf slaughtered 72 hours after being drenched with 100 mg./kg. of P^{32}-labeled ronnel had the following residues in the tissues: perirenal fat, 51 p.p.m.; omental fat, 55 p.p.m.; subcutaneous fat, 55 p.p.m.; bone marrow, 25 p.p.m.; muscle, 0.57 p.p.m.; and heart, 2.27 p.p.m.

American Cyanamid 12880.--One calf was given an oral dose of 10 mg./kg. of P^{32}-labeled Am. Cyanamid 12880, and another calf was given an intramuscular injection of 10 mg./kg. Both animals were slaughtered 7 days after treatment, and samples of tissues taken for residue studies. The analytical results are shown in table 3.

Four sheep were given intramuscular injections of 25 mg./kg. Two of the animals were slaughtered at 2 weeks and 4 weeks after treatment, and tissues were taken for analysis. The indicated residues after 2 weeks are shown in table 3. Significant residues were not found in any of the tissues.

Summary

Studies were conducted at Kerrville, Tex., to determine whether insecticides used on livestock or on pasture and forage crops will contaminate meat or milk, and if residues are produced, how long they will persist.

Single spray treatments of DDT, TDE, and methoxychlor applied at 0.5-percent concentrations to beef cattle caused storage of the insecticide in the fat. Lindane at a concentration of 0.03 percent could not be detected, but at a concentration of 0.075 percent both sprays and dips caused residues in the fat. Multiple-spray treatments with lindane and methoxychlor at 3-week intervals caused no greater storage in the fat than a single treatment. Multiple treatments at 2- or 3-week intervals with other chlorinated hydrocarbon insecticides--chlordane, gamma chlordane, DDT, dieldrin, heptachlor, TDE, Strobane, and toxaphene resulted in slight-to-moderate increases in the amount of storage. A 0.5-percent malathion spray applied at weekly intervals caused no storage in the fat.

When insecticides were fed to beef cattle and sheep as a contaminant of their feed at dosages likely to occur as residues on forage crops, all except methoxychlor were stored in the fat. The order of their storage was as follows: aldrin > dieldrin > heptachlor epoxide > BHC > DDT > chlordane > lindane > endrin > heptachlor > toxaphene.

All the chlorinated hydrocarbon insecticides, Co-Ral, and malathion were excreted in the milk of dairy cows following the spray treatments. Rotenone was not excreted in the milk and Co-Ral was present in very small amounts. Dieldrin caused the greatest contamination, followed in order by DDT, TDE, Dilan, toxaphene, Strobane, methoxychlor, Perthane, malathion, and Co-Ral. When Sevin, dicapthon, and Bayer 22408 were fed to dairy cows as a contaminant of their feed at dosages in excess of those likely to occur on forage crops, residues of these insecticides were not detected in the milk.

Acknowledgment

The authors express their appreciation to the Hercules Powder Company and to the Humble Oil and Refining Company for their cooperation in the toxaphene studies, and also to the former company for their corporation in the Delnav studies; to the B. F. Goodrich Chemical Company for their cooperation in the Strobane studies; to the Velsicol Corporation for their cooperation in the chlordane, gamma chlordane, and heptachlor studies; to the American Cyanamid Company for their cooperation in the malathion, dimethoate, and dicapthon studies; to Commercial Solvents Corporation for cooperation in the Dilan studies; to Rohm & Haas Company for cooperation in the Perthane studies; to Chemagro Corporation for cooperation on the Co-Ral studies; to Shell Chemical Company for their cooperation on the aldrin, dieldrin, and endrin studies; and to E. I. du Pont de Nemours and Company for cooperation in the methoxychlor studies.

Literature Cited

(1) Anderson, Charles A., Adams, James M., and McDougall, Daniel
1959. Photofluorometric method for the determination of Co-Ral residues in animal tissues. J. Agr. and Food Chem. 7: 256.

(2) Bann, James M., De Cino, Thomas J., Earl, Norman W., and Sun, Yun-Pei
1956. The fate of aldrin and dieldrin in the animal body. J. Agr. and Food Chem. 4: 937.

(3) Carter, R. H.
1947. Estimation of DDT in milk by determination of organic chlorine. Indus. and Engin. Chem., Analyt. Ed., 19: 54.

(4) __________ Wells, R. W., Radeleff, R. D., Smith, Charles L., Hubanks, P. E., and Mann, H. D.
1949. The chlorinated hydrocarbon content of milk from cattle sprayed for control of horn flies. J. Econ. Ent. 42: 116-118.

(5) Claborn, H. V., and Beckman, H. F.
1953. Determination of 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)-ethane in milk and fatty materials. Analyt. Chem. 24: 220.

(6) __________ Beckman, H. F., and Wells, R. W.
1951. Contamination of milk from DDT sprays applied to dairy barns. J. Econ. Ent. 43: 723.

(7) __________ Beckman, H. F., and Wells, R. W.
1950. Excretion of DDT and TDE in milk from cows treated with those insecticides for fly control. J. Econ. Ent. 43: 850-852.

(8) __________ Bowers, J. W., Wells, R. W., Radeleff, R. D., and Nickerson, W. J.
1953. Meat contamination from pesticides. Agr. Chem. 8(8): 37-39, 119-121.

(9) __________ Ivey, M. C., and Mann, H. D.
1960. A colorimetric method for the determination of Co-Ral in animal tissues. J. Econ. Ent. 53: 263-265.

(10) __________ and Wells, R. W.
1952. Methoxychlor, DDT, CS-708, and dieldrin--their rates of excretion in milk. Agr. Chem. 7(10): 28-29.

(11) __________ Radeleff, R. D., Beckman, H. F., and Woodard, G. T.
1956. Malathion in milk and fat from sprayed cattle. J. Agr. and Food Chem. 4: 941.

(12) Davidow, Bernard, and Woodard, Geoffrey
1949. An ultraviolet spectrophotometric method for the determination of benzene hexachloride. J. Assoc. Off. Agr. Chem. 32: 751.

(13) __________ and Radomski, J. S.
1953. Isolation of an epoxide metabolite from fat tissues of dogs fed heptachlor. J. Pharmacol. and Expt. Ther. 107: 259.

(14) Dunn, C. L.
1958. Determination of 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate). J. Agr. and Food Chem. 6: 203.

(15) Gannon, Norman, and Decker, G. C.
1958. The conversion of heptachlor to its epoxide on plants. J. Econ. Ent. 51: 3.

(16) Howell, D. W., Cave, H. W., Heller, V. G., and Gross, W. G.
1947. The amount of DDT found in the milk of cows following spraying. J. Dairy Sci. 30: 717-721.

(17) Hudy, J. A., and Dunn, C. L.
1957. Determination of organic chlorides and residues from chlorinated pesticides by combustion analysis. J. Agr. and Food Chem. 5: 351.

(18) Jackson, J. B., Ivey, M. C., Roberts, R. H., and Radeleff, R. D.
1959. Residue studies in sheep and goats dipped in 0.025% lindane. J. Econ. Ent. 52: 1030-31.

(19) Jacobson, M., Hornstein, I., and Murphy, R. T.
1959. The analysis of milk for rotenoids. J. Assoc. Off. Agr. Chem. 42: 174.

(20) Jones, L. R., and Riddick, J. A.
1951. Colorimetric determination of 2-nitro-1,1-bis(p-chlorophenyl)-alkanes. Analyt. Chem. 23: 349-351.

(21) ___________ and Riddick, J. A.
1952. Separation of organic insecticides from plant and animal tissues. Analyt. Chem. 24: 569-571.

(22) McGregor, W. S., Radeleff, R. D., Claborn, H. V., and Bushland, R. C.
1955. Dieldrin, aldrin and lindane. Systemic insecticides against livestock pests. Agr. Chem. 10: 34.

(23) Meyer, Charles F., Malina, Marshall A., and Polen, Percy B.
1960. Determination of heptachlor epoxide in fat and milk. J. Agr. and Food Chem. 8: 183-186.

(24) Norris, M. V., Easter, E. W., Fuller, L. T., and Kuchar, E. J.
1958. Colorimetric estimation of malathion residues in animal products. J. Agr. and Food Chem. 6: 111-114.

(25) O'Donnell, A. E., and Agazzi, E. J.
1952. Determination of aldrin in agricultural crops. Shell Chemical Co. Tech. Rpt. 197-52R.

(26) ___________, Neal, M. M., Weiss, F. T., Bann, J. M., De Cino, T. J., and Lan, S. C.
1954. Chemical determination of aldrin in crop materials. J. Agr. and Food Chem. 2: 573.

(27) ___________, Johnson, H. W., Jr., and Weiss, F. T.
1955. Chemical determination of dieldrin in crop materials. J. Agr. and Food Chem. 3: 757.

(28) Prickett, C. S., Kunze, F. M., and Laug, E. P.
1950. Modification of the Schechter method for the determination of methoxychlor or DDT in biological materials. J. Assoc. Off. Agr. Chem. 33: 880.

(29) Radeleff, R. D.
1950. Omentectomy of cattle for studying insecticide residues in the body. Vet. Med. 45: 125.

(30) __________ and Claborn, H. V.
1960. Excretion of Co-Ral in the milk of dairy cattle. J. Agr. and Food Chem. (in press).

(31) __________ Claborn, H. V., Wells, R. W., and Nickerson, W. J.
1952. Effects on beef cattle of prolonged treatment with a DDT spray. Vet. Med. 47: 94-96.

(32) Roberts, R. H., Radeleff, R. D., and Wheeler, H. G.
1960. Malathion residues in the tissues of sheep, goats and hogs. J. Econ. Ent. (in press).

(33) Schechter, Milton S., Pogorelskin, Milton A., and Haller, H. L.
1947. Colorimetric determination of DDT in milk and fatty materials. Indus. and Engin. Chem., Analyt. Ed., 19: 51-53.

(34) __________ and Hornstein, Irwin
1952. Colorimetric determination of benzene hexachloride. Analyt. Chem. 24: 544.

Table 1.--Parts per million of insecticide in the fat of animals at different times after a single spray treatment.

Insecticide	Animal	1 day	2 days	2 weeks	6 weeks	10 weeks	16 weeks	22 weeks	27 weeks
DDT 0.5%	Steer			5.2	5.1	2.1	1.7	2.2	1.1
				8.9	5.7	5.5	1.9	3.2	2.0
	Heifer			13.8	9.9	5.2	2.2	3.0	1.6
				16.9	11.9	8.4	3.4	3.1	2.1
			Av.	11.2	8.1	5.3	2.3	2.8	1.7
TDE 0.5%	Steer			7.8	3.7	3.4	0.5	0.5	0.4
				12.3	4.2	3.2	-	.9	.4
	Heifer			8.4	4.1	3.5	1.0	.5	.4
				15.6	9.0	5.5	1.9	.9	.7
			Av.	11.0	5.2	3.9	1.1	.7	.5
Methoxychlor 0.5%	Steer			2.4	1.8	0			
				2.4	0.8	-			
	Heifer			2.8	1.8	-			
				3.8	2.4	0			
			Av.	2.8	1.7				
Lindane 0.03%						None in either steers or heifers			
Delnav 0.25%	Hog	0	0.05						
	Steer	-	1.64						
	Sheep	-	0.12						
	Goat	-	.30						

Table 2.--Parts per million of lindane in the fat of cattle at different times after they were dipped or sprayed with 0.075-percent concentrations of the insecticide.

Treatment	1 week	2 weeks	3 weeks	4 weeks	6 weeks	8 weeks
Emulsion dip	23.0	-	4.9	-	1.4	-
	20.6	-	5.9	-	1.0	-
	-	14.2	-	1.5	-	0
	-	10.4	-	2.0	-	0
Suspension spray	10.4	-	1.3	-	0	-
	6.8	-	1.6	-	0.3	-
	-	6.4	-	1.7	-	0
	-	9.3	-	1.1	-	0
Emulsion spray	20.0	-	5.9	-	1.4	-
	14.6	-	3.4	-	0.4	-
		5.9	-	1.5	-	0.5
		6.4	-	0.9	-	.1
Control	0	-	0	-	0	-
	-	0	-	0	-	0

Table 3.--Apparent residues in tissues of calves and sheep treated with P^{32}-labeled insecticides.

Insecticide	Treatment (mg./kg.)	Slaughtered days after treatment	Parts per million of insecticide in:					
			Omental fat	Renal fat	Muscle	Liver	Kidney	Heart
			Calves					
Co-Ral	Spray							
	58	14	0.044	0.034	0.005	0.48	0.210	-
	25	7	.126	.102	.001	.348	-	-
	Oral							
	20	14	.005	.002	.020	.396	0.404	-
	10	7	.075	.004	.036	1.35	1.96	-
Dimethoate	Oral							
	10	7	0.012	0	0.029	1.13	0.99	0.267
	Intramuscular							
	10	7	0	0	.015	0.30	.18	.055
Delnav	Spray							
	16	7	1.25	0.86	0	0.16	0.06	0
			Sheep					
Dimethoate	Intramuscular							
	25	14	0	-	0.003	0.017	0.007	0.009
			0	-	.001	.011	.004	.003
	25	28	0	-	.012	.021	.013	.017
			0	-	.003	.010	.008	.009

Table 4.--Parts per million of insecticides in the fat of cattle after multiple spray treatments.

Insecticide	Spray interval (weeks)	Animal	After indicated spraying[1]						After last spraying		
			1st	2nd	3rd	4th	5th	6th	12 weeks	24 weeks	36 weeks
DDT 0.5%	3	Steer	16.2	-	-	33.9	-	-	6.3	-	2.9
			15.7	-	-	37.6	-	-	9.1	-	1.8
			-	32.4	-	-	-	-	-	4.9	-
		Heifer	22.1	-	-	27.0	-	-	7.8	-	1.9
			-	29.5	-	-	-	32.2	-	4.8	-
			-	31.6	-	-	-	38.2	-	4.4	-
		Av.	18.0	31.2	-	32.8	-	35.2	7.7	4.7	2.2
TDE 0.5%	3	Steer	8.4	-	-	30.6	-	-	9.2	-	-
			23.0	-	-	37.7	-	-	9.5	-	0.8
			-	36.6	-	-	-	-	-	1.2	-
		Heifer	8.4	-	-	41.2	-	-	22.3	-	0.8
			-	25.0	-	-	-	28.4	-	1.4	-
			-	36.6	-	-	-	28.4	-	1.2	-
		Av.	13.2	32.7	-	36.5	-	28.4	13.6	1.3	0.8
Methoxychlor 0.5%	3	Heifer	1.6	-	-	0.8	-	-	0		
			1.8	-	-	.6	-	-	0		
			1.0	-	-	.9	-	-	0		
		Steer	-	0.8	-	-	-	1.6			
			-	1.4	-	-	-	2.1			
			-	2.2	-	-	-	3.4			
		Av.	1.5	1.5	-	0.8	-	2.4			
Lindane 0.03%	3	Steer	None after any spraying								
Delnav 0.15%	2	Cattle[2]	0.12	0.05	0.21	-	-	0.13			

Table 4.--Continued.

Insecticide	Spray interval (weeks)	Animal	After indicated spraying[1]						After last spraying	
			1st	2nd	3rd	4th	5th	6th		
									11 weeks	28 weeks
Dieldrin 0.05%	3	Heifer	8	10	21	27	-	-	-	6
			7	-	15	23	-	-	-	6
			6	9	14	22	-	-	17	6
		Av.	7	10	16	24	-	-	17	6
									8 weeks	16 weeks
Heptachlor 0.5%	2	Steer	9.2	-	11.9	-	15.0	16.7	11.2	0
			-	10.2	-	16.6	-	15.4	-	-
		Heifer	13.3	-	16.0	-	20.4	19.2	14.9	2.2
			-	17.1	-	21.6	-	26.1	20.5	3.8
		Av.	11.2	13.6	13.9	19.1	17.7	19.3	15.5	2.0
Chlordane 0.5%	2	Steer	-	-	-	-	-	13.6	5.7	1.5
			-	-	-	-	-	34.4	11.9	7.6
		Heifer	-	-	-	-	-	18.9	7.8	-
			-	-	-	-	-	15.8	6.6	4.5
		Av.	-	-	-	-	-	20.7	8.0	4.5
	2	Steer[3]	-	-	-	-	-	34.0	-	7.5
			-	-	-	-	-	-	-	2.4
		Heifer[3]	-	-	-	-	-	22.0	-	1.7
		Av.	-	-	-	-	-	28.0	-	3.9
Gamma chlordane 0.5%	2	Steer	-	7.0	-	14.8	-	22.0	15.2	0
			11.9	-	14.7	-	18.0	-	-	0
		Heifer	-	13.0	-	19.5	-	26.8	16.1	0
			5.5	-	16.9	-	18.4	23.2	-	1.1
		Av.	8.7	10.0	15.8	17.1	18.2	24.0	15.6	-

									6 weeks	10 weeks	14 weeks
Strobane 2%	2	Steer	-	-	-	-	-	32.4	9.1	3.4	3.3
			-	-	-	-	-	33.9	14.4	6.0	4.1
			-	-	-	-	-	20.4	4.6	1.2	0.8
		Heifer	-	-	-	-	-	32.2	10.2	5.1	3.9
			-	-	-	-	-	23.6	6.9	3.0	1.6
			-	-	-	-	-	-	6.9	4.0	1.9
		Av.	-	-	-	-	-	28.5	8.7	3.8	2.6

1/ Fat samples were taken at the end of the intervals between spraying.

2/ Composite sample from three animals.

3/ Cattle were in poor condition.

Table 5.--Parts per million of toxaphene in fat of calves sprayed with 0.5-percent toxaphene.[1]

Animal	2 weeks after indicated spraying[2]										After 12th spraying	
	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	4 weeks	6 weeks
						Emulsion prepared in laboratory						
Steer	0	-	7	-	7	-	-	10	-	13	-	-
	-	11	-	0	-	-	-	-	-	9	-	-
	-	3	-	0	-	10	-	-	10	-	-	0
	2	-	-	-	-	-	-	-	-	-	-	-
	7	-	17	-	-	8	-	-	12	-	-	5
	-	2	-	4	-	-	7	-	-	-	5	-
Av.	3	5	12	1	7	9	7	10	11	11	5	2
						Emulsion from concentrate furnished by Humble Oil and Refining Company						
Steer	2	-	9	-	-	-	-	9	-	13	-	-
	-	3	-	4	-	-	12	-	-	20	-	-
	-	11	-	5	-	9	-	-	6	-	-	2
	0	-	13	-	7	-	-	-	-	-	-	-
	0	-	7	-	-	7	-	-	8	-	-	6
	-	-	-	8	-	-	-	-	-	-	4	-
Av.	1	7	10	6	7	8	12	9	7	16	4	4

---

[1] All samples taken from the control animals were negative or within the experimental error of the methods, which is ± 4 p.p.m.

[2] None found in any sample before the third spraying.

Table 6.--Parts per million of Strobane in fat of cattle sprayed with 0.5-percent Strobane emulsion or suspension.

Animal	2 weeks after indicated spraying							6 weeks after last spraying
	1st	2nd	4th	6th	8th	10th	12th	
				Emulsion				
Steer	-	-	-	-	3.7	-	6.9	-
	-	-	7.2	-	4.4	-	6.5	-
	-	3.8	-	7.3	-	-	6.3	-
Heifer	-	6.4	-	8.8	-	6.9	4.8	1.4
	1.8	-	-	-	-	-	5.9	2.6
	3.8	-	-	-	-	-	9.4	4.3
Av.	2.8	5.1	7.2	8.0	4.0	6.9	6.6	2.8
				Suspension				
Steer	-	-	7.8	-	5.1	-	4.7	-
	-	-	6.4	-	6.8	-	6.5	-
	-	6.1	-	6.9	-	9.6	7.5	3.1
Heifer	-	5.6	-	9.5	-	5.5	5.0	-
	3.8	-	-	-	-	-	7.1	-
	1.9	-	-	-	-	-	5.0	2.0
Av.	2.8	5.8	7.1	8.2	6.0	7.5	5.9	2.6

Table 7.--Parts per million of insecticides stored in the fat of cattle and sheep that had known amounts added to their diet.

Insecticide	Dosage (p.p.m.)	Animal	After feeding				After feeding ceased				
			4 weeks	8 weeks	12 weeks	16 weeks	4 weeks	8 weeks	20 weeks	32 weeks	36 weeks
Aldrin	25	Steer	58	79	-	-	46	36	19	9	-
			41	77	-	-	56	36	21	7	-
		Av.	50	78	-	-	51	36	20	8	-
		Wether	60	77	-	-	-	46	25	31	33
		Ewe	60	79	-	-	67	30	25	14	11
		Av.	60	78	-	-	67	38	25	23	22
	10	Ewe	-	47	51	57					
			-	52	52	52					
			38[1]	62	36	47					
		Wether	-	45	48	57					
			-	46	-	59					
			-	64	50	59					
		Av.	38	53	49	55					
		Heifer	34	46	39	59					
			29	48	51	58					
			30	38	41	41					
			37	35	48	52					
			36	41	41	38					
		Av.	35	41	44	49					
	5	Ewe	11	17	14	13					
			8	11	21	20					
			7	8	-	15					
		Wether	4	14	14	9					
			5	11	20	18					
			6	7	-	19					
		Av.	7	11	17	17					

Tech. aldrin 60%	5	Ewe	0	0	6	6			
			0	3	5	9			
			0	0	3	6			
		Wether	0	1	4	6			
			0	0	2	5			
			-	2	3	-			
		Av.	0	1	4	6			
		Heifer	0	2	4	5			
			0	-	8	-			
			2	3	11	3			
		Steer	0	6	-	7			
			0	4	10	8			
		Av.	0	4	8	6			
	2.5	Wether	4.6	-	5.6	5.3	-	2.6	
			-	4.7	-	4.7	-	3.2	
		Ewe	4.2	-	3.4	3.9	-	1.5	
			-	4.7	-	4.2	-	3.5	
		Av.	4.4	4.7	4.5	4.5	-	2.8	
		Steer	3.9	-	-	5.9	-	1.8	
			-	5.7	-	6.5	-	2.1	
		Heifer	3.6	-	4.7	4.2	-	2.0	
			-	3.5	-	4.3	-	0.9	
		Av.	3.8	4.6	4.7	5.2	-	1.7	
BHC	100	Steer	117	228	227	257	94	-	12
			117	-	252	254	72	-	19
			187	242	259	248	90	-	14
		Heifer	137	213	220	228	95	-	24
			122	209	194	264	71	-	14
		Av.	159	223	230	250	84	-	17

Table 7.--Continued.

Insecticide	Dosage (p.p.m.)	Animal	After feeding				After feeding ceased				
			4 weeks	8 weeks	12 weeks	16 weeks	4 weeks	8 weeks	20 weeks	32 weeks	36 weeks
									16 weeks		
BHC	100	Ewe	82	-	101	104	-	28	3		
			144	140	138	127	-	43	5		
			98	114	108	101	-	24	6		
		Wether	112	115	113	110	-	-	-		
			125	105	122	135	-	30	-		
			113	117	131	128	-	35	5		
		Av.	112	118	119	117	-	32	5		
									20 weeks		
Chlordane	25	Steer	9	18	-	-	16	5	0		
		Heifer	16	19	-	-	11	5	0		
		Av.	12	18	-	-	14	5	-		
		Wether	4	12	-	-	0				
		Ewe	11	13	-	-	0				
		Av.	8	12	-	-	0				
	10	Ewe	-	19	17	7					
			-	15	9	6					
		Wether	9	16	17	15					
			-	13	10	7					
			-	20	27	-					
		Av.	9	16	16	9					
		Heifer	8	12	9	-					
			11	15	10	9					
			12	12	10	10					
			-	15	11	17					
			13	11	10	9					
		Av.	11	13	10	11					

									16 weeks	24 weeks	
DDT	25	Steer	-	29	-	38	11	-	-	4.5	
			28	-	45	38	23	-	7.3	3.9	
		Heifer	-	40	-	46	26	-	12.0	6.8	
			15	-	39	37	16	-	13.7	7.6	
		Av.	22	34	42	40	19	-	11.0	5.7	
		Ewe	5	-	15	15	10	-	6.1	3.9	
			-	10	-	13	7	-	6.1	3.2	
		Wether	15	-	14	16	10	-	5.4	3.2	
			-	15	-	17	8	-	4.5	2.7	
		Av.	10	12	14	15	9	-	5.5	3.2	
	10	Cattle (2)	6.8								
		Sheep (2)	3.1								
									20 weeks	32 weeks	
Dieldrin	25	Steer	70	63	-	-	68	55	25	10	
		Heifer	80	86	-	-	67	36	15	9	
		Av.	75	74	-	-	68	46	20	10	
		Wether	39	70	-	-	58	37	32	33	31
		Ewe	48	68	-	-	43	45	32	-	10
		Av.	44	69	-	-	50	41	32	33	20
									16 weeks	24 weeks	
	10	Steer	-	29	-	48	29	-	19	9	
			18	-	37	45	22	-	16	8	
		Heifer	-	22	-	42	13	-	11	5	
			14	-	33	39	15	-	13	9	
		Av.	16	26	35	44	20	-	15	8	
		Wether	-	65	-	60	37	-	28	12	
			8	-	38	40	20	-	20	12	
			-	23	-	41	35	-	25	19	
			9	-	31	52	29	-	22	15	
		Av.	8	44	34	48	30	-	24	14	

Table 7.--Continued.

Insecticide	Dosage (p.p.m.)	Animal	After feeding				After feeding ceased				
			4 weeks	8 weeks	12 weeks	16 weeks	4 weeks	8 weeks	20 weeks	32 weeks	36 weeks
Dieldrin	2.5	Wether	5.7	-	8.6	9.8	9.8				
			-	7.5	-	10.2	8.5				
		Ewe	7.7	-	11.0	10.4	9.7				
			-	4.6	-	11.5	7.0				
		Av.	6.7	6.0	9.8	10.5	8.8				
		Steer	6.9	-	9.4	11.3	5.2				
			-	13.4	-	14.8	7.3				
		Heifer	7.1	-	11.1	12.3	4.4				
			-	10.5	-	18.9	6.0				
		Av.	7.0	11.4	10.2	14.3	5.6				
	1	Steer	4.2	-	6.0	-	1.9				
			-	5.3	-	5.5	2.4				
		Av.	4.2	5.3	6.0	5.5	2.2				
		Wether	-	3.9	-	-	2.1				
		Ewe	1.8	-	2.0	2.6	2.4				
		Av.	1.8	3.9	2.0	2.6	2.2				
Endrin	5	Steer	1.4	-	2.5	1.9					
			-	2.2	-	-					
		Heifer	1.2	-	2.4	1.3					
			-	0.8	-	3.6					
		Av.	1.3	1.5	2.4	2.3					
		Wether	1.9	-	0.5	3.5					
			-	1.5	-	1.4					
		Ewe	1.1	-	1.2	1.7					
			-	2.4	-	-					
		Av.	1.5	2.0	0.8	2.2					

	2.5	Steer	0.9	-	0.4	1.6	
			-	2.8	-	1.0	
		Heifer	1.6	-	1.3	0	
			-	2.3	-	0.6	
		Av.	1.2	2.6	0.8	0.8	
		Wether	3.4	-	2.8	0.9	
			-	1.8	-	0.3	
			-	-	-	1.6	
			3.1	-	1.8	2.7	
		Av.	3.2	1.8	2.3	1.4	
Heptachlor	10	Steer	5	-	5	4	1
			3	-	2	2	0
		Heifer	-	-	-	-	2
			-	8	-	9	4
		Av.	4	8	3.5	7.5	1.7
		Ewe	-	8	-	6	6
			6	-	-	10	-
		Wether	-	9	-	9	6
			5	-	8	7	3
		Av.	5.5	8.5	8	8	5
	2.5	Steer	1.5	-	0	0.5	
			-	0.9	-	-	
		Heifer	1.2	-	1.4	0	
			-	0.5	-	0	
		Av.	1.4	0.7	0.7	0.2	
		Wether	0.9	-	0	2.0	
			0	0	-	0.4	
		Ewe	1.2	-	0	3.4	
			-	0	-	2.6	
		Av.	1.0	0	0	2.1	

Table 7.--Continued.

Insecticide	Dosage (p.p.m.)	Animal	After feeding				After feeding ceased				
			4 weeks	8 weeks	12 weeks	16 weeks	4 weeks	8 weeks	20 weeks	32 weeks	36 weeks
Methoxychlor	25	Steer	-	0	-	0					
			0	-	-	0					
		Heifer	-	0	-	0					
		Av.	0	0	-	0					
		Ewe	-	0	-	0					
			0	-	0	0					
		Wether	-	0	-	0					
			0	-	0	0					
		Av.	0	0	0	0					
Toxaphene	100	Ewe	29	30	-	30	19	0			
			21	19	-	21	9	0			
			19	18	25	19	11	0			
		Wether	20	17	-	17	10	0			
			25	19	-	19	15	0			
			16	24	0	17	10	3			
		Av.	22	21	25	20	12	0.5			
		Steer	25	27	36	37	10	-			
			-	45	43	52	29	9			
			30	34	29	29	24	0			
		Heifer	23	27	25	33	10	3			
			26	35	33	39	15	0			
		Av.	26	34	33	38	14	3			

	25	Ewe	1	1	3	11			
			1	1	3	7			
			0	1	1	11			
		Wether	1	0	9	-			
			2	4	1	4			
			4	4	1	6			
		Av.	2	2	3	8			
		Heifer	2	4	11	16			
			3	4	7	12			
			1	9	9	16			
		Steer	4	4	11	8			
			1	1	12	9			
		Av.	2	4	10	12			
	10	Cattle (2)	4						
		Sheep (2)	4						
Lindane[2]	1	Heifer	No fat	1.0	1.3	1.6	-	0.9	-
		Steer	0.3	0.8	2.0	0.4	0.5	-	0.6
	10	Heifer	3.5	6.9	7.6	2.0	-	0.6	-
		Steer	No fat	6.7	8.3	4.2	4.9	-	-
	100	Heifer	59.0	76.0	86.0	40.0	-	3.7	1.0
		Steer	70.0	76.0	111.0	60.0	12.0	-	1.3

---

[1] Composite sample made up of equal amounts of six individual samples.

[2] These analyses were made by chemists of Hooker Electrochemical Co. The experiment has been described by Radeleff (29).

Table 8.--Parts per million of dieldrin in the tissues of steers, sheep, and hogs fed varying levels of aldrin in the diet for a period of 12 weeks.[1]

Dosage (p.p.m.)	P.p.m. of dieldrin in--				
	Body fat	Renal fat	Liver	Kidney	Muscle
Steers					
0	0	0	0	0	0
0	0	0	0	0	0
0.25	0.99	0.88	0.05	0.02	0
.25[2]	.68	.57	.05	0	0
.75	3.40	2.90	.25	0.38	0.07
.75[2]	2.10	1.80	.10	.26	0
2.00	8.50	7.80	.66	.63	0.13
2.00	5.10	4.20	.33	.28	.12
10.0	39.2	44.5	3.84	3.50	.72
10.0	17.8	19.2	0.93	1.32	.17
Sheep					
0	0	0	0	0	0
0[2]	0	0	0	0	0
0.25	0.62	0.65	0.09	0.06	0.02
.25[2]	.28	.33	.04	0	0
.75	2.03	1.90	.14	0.16	0.03
.75[2]	1.64	1.50	.13	.08	.03
10.0	42.3	41.5	3.14	1.36	.73
10.0	19.0	16.4	1.29	0.60	.26
Hogs					
0	0	0	0	0	0
0[2]	0	0	0	0	0
0.25	0.55	0.53	0.03	0	
.25[2]	.12	.10	0	0	
.75	1.66	1.40	0.06	0.05	
.75[2]	0.50	0.55	0	0	
10.0	17.4	19.8	0.77	0.69	
10.0	4.20	4.0	.19	.48	

---

[1] The control samples were given a theoretical value of 0. The blanks from the control samples were subtracted from the test samples in the same group. The other zeros in the table indicate values less than 0.02 p.p.m.

[2] Animals slaughtered 6 weeks after the end of the 12-week feeding period; all others were slaughtered at the end of the feeding period.

Table 9.--Parts per million of heptachlor epoxide in the fat of cattle fed different dosages of heptachlor in the diet.

Dosage (p.p.m.)	Weeks of feeding	Heptachlor epoxide in fat (p.p.m.)
3.75	16	2.7
7.5	10	2.9
15	8	6.1
30	8	13.8
30	10	16.1
60	8	34.1
60	10	38.8
60	14	59.8
60	16	61.9

Table 10.--Parts per million of heptachlor epoxide in the fat of cattle fed heptachlor or heptachlor epoxide in the diet.

Insecticide	Dosage (p.p.m.)	Animal	After feeding		After feeding ceased	
			4 weeks	8 weeks	4 weeks	8 weeks
Heptachlor	3.75	Steer	0.80	1.53	1.03	0.85
		Heifer	.54	1.11	0.97	-
		Av.	0.67	1.32	1.00	0.85
Heptachlor epoxide	1.0	Steer	2.04	5.08	3.07	2.86
		Heifer	1.65	3.33	2.02	1.95
		Av.	1.84	4.20	2.54	2.40
	3.75	Steer	7.51	15.4	12.7	7.5
		Heifer	7.32	13.3	7.6	5.5
		Av.	7.42	14.4	10.2	6.5

Table 11.--Parts per million of DDT in milk from three cows receiving one treatment with a 0.5-percent suspension.

Days	Cow No. 1	Cow No. 2	Cow No. 3	Average
Before spraying	0.1	0.2	0.1	
After spraying				
2	3.8	3.0	1.8	2.8
7	1.1	2.0	1.2	1.4
14	0.5	0.5	1.0	0.7
21	.4	.4	0.9	.6

Table 12.--Parts per million of dieldrin and methoxychlor in milk from cows sprayed with 0.5-percent of these insecticides.

Days	Dieldrin emulsion	Methoxychlor	
		Emulsion	Suspension
Before spraying	0	0	0
After spraying			
1	2.9	0.70	0.25
2	5.5	.48	.44
3	7.0	.37	.33
4	3.8	-	-
5	-	0.18	0.19
7	1.7	.09	.16
10	-	.06	.05
14	1.3	.06	.05
22	0.4	0	0
Resprayed			
1	-	0.33	0.30
2	-	.40	.38
3	-	.30	.33
5	-	.18	.15
7	-	.09	.14
10	-	.06	.06
14	-	0	.06
21	-	0	0
Av.	3.2	0.20	0.18

Table 13.--Parts per million of Dilan in milk from two cows sprayed with a 0.5-percent suspension.

Days	Cow No. 1	Cow No. 2	Average
Before spraying			
1	0	0	-
Same day	0.06	0.07	-
After spraying			
1	0.45	0.77	0.61
2	.70	.80	.75
3	.52	.83	.68
7	.92	.26	.59
10	.74	.33	.54
14	.62	.44	.53
Resprayed			
1	0.64	0.65	0.64
2	.96	1.26	1.11
3	.90	1.01	.96
7	1.07	0.92	1.05
10	0.52	.47	0.50
14	.40	.30	.35
21	.20	.20	.20

Table 14.--Parts per million of Perthane in milk from six cows sprayed with 0.5-percent of Perthane.

Days after spraying	Emulsion				Suspension			Oil solution	
	Cow No. 1		Cow No. 2		Cow No. 3		Cow No. 4	Cow No. 5	Cow No. 6
	Kerrville	Rohm & Haas	Kerrville	Rohm & Haas	Kerrville	Rohm & Haas	Rohm & Haas	Rohm & Haas	Rohm & Haas
1	0.07	0.06	0.17	0.04	0.25	0.11	0.08	0	0
2	.10	.04	.18	.15	.05	.08	.22	0.90	0.10
3	.06	.12	.36	.22	.10	.07	.16	.08	.18
5	0	.13	.17	.28	.04	.12	.13	.10	.11
7	0	.12	.14	.07	.14	.04	.11	.05	.18
14	0	.17	.22	.02	.17	.04	.10	.13	.08
21	0	.01	.32	0	.12	.03	.03	.10	.14
Resprayed									
1	0	0.02	0.31	0.01	0.25	0.13	0.06	0.09	0.13
2	0	.11	.39	.03	.22	.08	.16	.11	.04
3	0	.11	.26	.04	.32	.03	.12	.03	.05
5	0.06	.03	.38	.04	.06	.10	.14	0	.04
7	.08	.04	.12	.04	0	.09	.02	0.10	.07
14	0	.01	.12	.02	0.05	.07	.05	.07	.12
21	0	0	0	.02	0	.05	0	0	.06
Average	0.03	0.07	0.22	0.07	0.13	0.07	0.10	0.07	0.09

Table 15.--Parts per million of Strobane and toxaphene in milk from cows sprayed twice at 3-week intervals with 0.5-percent of these insecticides.

Days	Strobane				Toxaphene			
	Emulsion		Suspension		Emulsion		Suspension	
	Cow No. 1	Cow No. 2	Cow No. 3	Cow No. 4	Cow No. 1	Cow No. 2	Cow No. 3	Cow No. 4
Before spraying	0	0	0	0	0	0	0	0
After first spray								
1	0.61	0.87	0.45	0.73	0.69	0.51	0.72	0.56
2	.52	.70	.53	.69	.67	.55	.66	.82
3	.36	.45	.44	.49	.50	.37	.60	.56
5	.24	.17	.17	.24	.23	.23	.30	.23
7	.07	.07	.16	.13	.19	.13	.28	.11
14	.13	0	.08	0	.07	.06	.25	0
21	0	.10	.10	0	.08	.07	.21	0.03
After second spray								
1	0.47	0.46	0.57	0.45	0.47	0.57	0.89	0.54
2	.55	.55	.69	.66	.51	.59	.92	.70
3	.29	.45	.53	.52	.42	.40	.66	.48
5	.13	.22	.40	.38	.24	.25	.40	.22
7	.04	.08	.17	.16	.15	.16	.20	.15
14	0	.02	.03	.04	.09	.10	.14	.02
21	0	.04	.02	0	.06	.05	.04	0

Table 16.--Parts per million of Strobane and toxaphene in milk of cows sprayed twice daily with 1 ounce of 2-percent oil solutions of Strobane and toxaphene

Days	Strobane		Toxaphene	
	Cow No. 5	Cow No. 6	Cow No. 11	Cow No. 12
After spraying started				
1	-	-	0.11	0.13
3	0.26	0.30	.32	.50
7	.27	.39	.30	.40
14	.30	.35	.20	.31
21	.30	.33	.25	.34
After spraying ceased				
7	0.08	0.06	0.09	0.09
14	0	.02	.10	.10
21	-	-	.07	.06

Table 17.--Apparent residues of Co-Ral in milk from cows sprayed with 0.5% and 0.75% P^{32}-labeled Co-Ral.

Time after spraying	Apparent Co-Ral (p.p.m.) in milk	
	0.5%	0.75%
5 hours	0.196	0.245
1 day	.126	.158
1 day, 8 hours	.121	.154
2 days	.064	.082
2 days, 8 hours	.069	.082
3 days	.037	.063
3 days, 8 hours	.034	.064
4 days	.034	.036
5 days	.019	.024
7 days	.012	.014
11 days	.001	.003

Table 18.--Toxaphene in milk from cows fed varying levels of toxaphene in the diet.

Dosage (p.p.m.)	Cow No.	Toxaphene in milk (p.p.m.)										
		After feeding (weeks)								Weeks after feeding ceased		
		1	2	3	4	5	6	7	8	1	2	3
20	3	0.17	0.24	0.24	0.31	0.29	0.33	0.25	0.21	0.10	0.01	-
	4	.26	.31	.31	.41	.34	.42	.31	.25	.06	.04	-
	5	.16	.24	.24	.35	.35	.35	.26	.24	.06	.02	-
	Av.	0.20	0.26	0.26	0.36	0.33	0.37	0.27	0.23	0.07	0.02	-
60	6	0.61	0.65	0.74	0.70	0.67	0.68	0.47	0.44	0.08	0.05	0.04
	7	.61	.69	.87	.66	.69	.77	.53	.52	.14	.11	.09
	8	.47	.50	.65	.67	.53	.69	.48	.48	.16	.13	.09
	Av.	0.56	0.61	0.75	0.68	0.63	0.71	0.49	0.48	0.13	0.10	0.07
100	9	0.90	0.99	0.92	1.06	0.87	0.96	0.93	0.90	0.11	0.05	0.08
	10	.87	1.00	1.08	1.19	1.13	1.04	.97	.96	.18	.16	.15
	11	.85	1.05	1.04	1.19	0.92	0.89	.68	.88	.17	.18	-
	Av.	0.87	1.01	1.01	1.15	0.97	0.96	0.86	0.91	0.15	0.13	0.12
140	12	1.46	1.56	1.68	1.75	1.31	1.39	1.36	1.52	0.19	0.17	0.12
	13	1.13	1.09	1.40	1.45	1.23	1.23	1.53	1.44	.30	.22	.21
	14	1.74	2.36	2.32	2.47	1.96	2.31	2.24	2.51	.46	.80	.26
	Av.	1.44	1.67	1.80	1.89	1.50	1.64	1.71	1.82	0.32	0.40	0.20
Control	1	0.00	0.02	0.00	0.07	0.20	0.17	0.00	0.00	0.00	0.00	0.00
	2	.00	.00	.00	.04	.11	.16	.00	.00	.00	.00	.00
	Av.	0.00	0.01	0.00	0.06	0.16	0.16	0.00	0.00	0.00	0.00	0.00